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(54) Title: METHOD OF MODIFYING THE PROPERTIES OF A SUBSTRATE SURFACE BY COVALENT BONDING OF A COMPOUND TO THE SURFACE, AND MEMBRANE MODIFIED ACCORDING TO THIS METHOD

(57) Abstract

A method of modifying the properties of a surface of a substrate by covalent bonding of a compound to the surface, the compound comprising one or more functional groups which are all or partly activated by a reagent in a solvent-containing first solution, whereafter the activated compound is bonded to a functional substrate surface in a solvent-containing second solution.

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Title: Method of modifying the properties of a substrate surface by covalent bonding of a compound to the surface, and membrane modified according to this method.

The invention relates to a method of modifying a substrate surface by covalent bonding of a compound to the substrate surface, so that the properties of the substrate surface with regard to the interaction of the substrate surface with chemical compounds and/or cells in particular environments are modified.

An example of such a substrate and method is known from Dutch Patent Application No. 8701337, according to which a physiologically active compound is bonded to the substrate surface via a polyacid, which changes the properties of the substrate surface, when in contact with blood, in such a way that less or no coagulation occurs on the substrate surface.

The object of the invention is to obtain a new, simple method with which the properties of substrate surfaces, preferably substrate surfaces with a suitable functionality, can be modified. The existing methods typically start from activating the substrate surface, followed by immobilizing a particular compound on the activated substrate surface. When such methods are used on specific substrate surfaces there is a chance of undesired modification of the substrate surface. In the method according the invention, the order is reversed, i.e. first the compound is activated and then it is bonded to the substrate surface. The advantage thereof is that the substrate surface is not available to the reagent used for activating the compound.

The invention is characterized in that the compound to be bonded contains one or more functional groups, for example, hydroxyl- and/or carboxyl- and/or amino groups; the compound is activated by means of a reagent in a solvent suitable for this purpose; and via a one-step procedure the activated compound is bonded covalently to a substrate surface with a suitable functionality. Preferably, in the method according to

- not plasma

the invention, a substrate surface is selected which comprises functional groups necessary for the covalent bonding of the activated compound, preferably hydroxyl- and/or carboxyl- and/or amino groups. To immobilize the compound on the substrate surface, preferably a bifunctional reagent is used, more preferably a carbonylating reagent of the general structural formula $R_1-(CO)-R_2$, wherein R_1 and R_2 represent groups which can easily be separated. Examples of such a reagent are 1,1'-carbonyl diimidazole or 1,1-carbonyldi-1,2,4-triazole. The choice of the reagent is determined *inter alia* by the nature of the functional group(s) of the compound and of the substrate and by the choice of the solvents to be used.

Apart from the feature that the compound contains one or more functional groups, the compound can be any compound which, after immobilization, changes the properties of the substrate surface involved, when in contact with a particular environment. Examples are compounds which, after immobilization, increase the biocompatibility of the substrate surface, for instance heparin.

The method is particularly suitable when the compound is a polyelectrolyte or a polyelectrolyte-containing compound having more than one functional group, for instance heparin. With these compounds, during activation of the compound, no cross-linking of the compound occurs, which adds an extra advantage to the method.

The compound is activated in a solvent suitable for activation, which solvent is not reactive with respect to the bifunctional reagent and the activated compound. The compound is immobilized in a solvent suitable for the immobilization of a sufficient amount of the compound to be immobilized. Examples of suitable solvents for activation in the case where the bifunctional reagent is a carbonylating reagent of the general structural formula $R_1-(CO)-R_2$, for example, 1,1'-carbonyl diimidazole or 1,1-carbonyl-1,2,4-triazole, are dioxane, tetrahydrofuran, acetone, formamide, dimethyl formamide and dichloromethane. The choice of the solvent

during activation of the compound is also based on the solubility of the compound. The choice of the solvent during immoblization is also determined by the interaction of the substrate surface with the solvent and by the solubility of the activated compound in the solvent. Mixtures of several solvents can also be used.

By way of example, the compound can be activated in a solution with a solvent of an organic nature, which solution, after the required reaction time, is added to the substrate surface which is in an aqueous environment.

As regards the compound, it may be necessary to modify it in such a way that its solubility in the selected solvent is increased. If the compound is a polyelectrolyte, this can be done by an appropriate choice of the counterion.

When a substrate surface to be modified does not have the required reactive groups it is possible to provide the surface in question with such groups beforehand.

In order to optimize the interaction of the compound after immobilization with components from the environment to which the substrate is exposed, it is possible to provide the surface beforehand with so-called "spacer" molecules, which increase the distance between the substrate surface and the compound. An example of a suitable spacer molecule is a functional poly(ethylene oxide)-containing molecule which, after bonding to the substrate surface, contains one or more fucntional groups, preferably a hydroxyl- and/or carboxyl- and/or amino group(s).

In addition to the advantage realized by the invention, namely obtaining a substrate surface with modified properties, a further advantage is that by first activating the compound there is no bifunctional reagent available during the immobilization step to react with the substrate surface. This makes the method advantageously suitable for modifying hydrophilic substrates, of which only the properties of the substrate surface change upon modification according to the

method. Examples of such hydrophilic substrates are cellulose-based membranes and hydrogels.

The method also has the advantage that when the compound to be bonded is a polyelectrolyte-containing compound having more groups per molecule which are reactive with respect to the bifunctional reagent, for example heparin, cross-linking of the polyelectrolyte does not occur during the activation of said polyelectrolyte. The advantage is that the activity of the compound is retained after immobilization. Furthermore, the method is so simple that the surface modification with the activated compound can be carried out as a one-step procedure.

The invention also relates to a membrane having a surface modified in accordance with the method of the invention, preferably a cellulose membrane wherein the compound to be activated is heparin or a heparin-analogue.

In accordance with a further elaboration of the invention, hydroxyl- and/or carboxyl groups of the heparin are first activated with a bifunctional reagent, preferably a carbonylating reagent of the general structural formula $R_1-(CO)-R_2$, more preferably 1,1'-carbonyl diimidazole or 1,1-carbonyl-1,2,4-triazole. The activation takes place in a solvent which is not reactive with respect to the bifunctional reagent and the activated heparin, for example formamide. During this activation no cross-linking of the heparin occurs. Subsequently, the membrane is incubated in the solution of the activated heparin. After rinsing and drying the heparinized membrane is obtained.

In addition to the advantage accomplished by the invention, namely a strong improvement of the blood compatibility of the membrane, a further advantage is that by pre-activating the heparin with the reagent, during the immobilization step the reagent is not available for cross-linking the membrane, so that the permeability of the membrane involved is not affected by cross-linking of the membrane by the bifunctional reagent.

EXAMPLE

Heparin sodium salt is converted into heparin-benzyl trimethyl ammonium salt via an ion-exchange procedure in order to increase the solubility of heparin in organic solvents. Subsequently, the heparin salt is dissolved in formamide (0.25 $g.ml^{-1}$) to which carbonyl diimidazole (CD1) is added until a heparin/CD1 ratio (w/w) of 8.5 has been reached. After stirring for half an hour at room temperature, a cellulose membrane is added whereafter the reaction mixture is regularly homogenized for 48 hours. Thereafter, the membrane is rinsed with formamide, water and a NaCl solution and subsequently incubated in a glycerol solution and dried.

The heparinized membrane has a typical heparin surface concentration of about 50 $\mu g.cm^{-2}$. This amount can be varied controllably by adjusting the heparin concentration in the reaction mixture (see Fig. 1) The heparinized membrane has the same permeability to urea as the non-modified membrane. The blood compatibility of the membrane has been highly improved by the heparinization procedure. In comparison with citrate plasma which is incubated in non-modified membranes, citrate plasma which is incubated in the heparinized membranes shows a prolonged coagulation time (see Fig. 2). Further, heparinized membranes, unlike non-modified membranes, hardly, if at all, give rise to complement activation when they are contacted with blood (see Fig. 3).

CLAIMS

1. A method of modifying the properties of a surface of a substrate by covalent bonding of a compound to the surface, characterized in that the compound contains one or more functional groups which are all or partly activated by a reagent in a solvent-containing first solution, whereafter the activated compound is bonded to a functional substrate surface in a solvent-containing second solution.

2. A method as claimed in claim 1, characterized in that the functional reagent is a bifunctional reagent.

3. A method as claimed in claim 2, characterized in that the bifunctional reagent is a carbonylating reagent.

4. A method as claimed in claim 3, characterized in that the carbonylating reagent has the structural formula R_1-(CO)-R_2, wherein R_1 and R_2 represent groups which can easily be separated.

5. A method as claimed in claim 4, characterized in that the carbonylating reagent is 1,1'-carbonyl diimidazole.

6. A method as claimed in claim 4, characterized in that the carbonylating reagent is 1,1'-carbonyl-1,2,4-triazole.

7. A method as claimed in claims 1-6, characterized in that the functional groups of the compound are carboxyl- and/or hydroxyl- and/or amino groups.

8. A method as claimed in claim 1, characterized in that the functional substrate surface comprises hydroxyl- and/or carboxyl- and/or amino groups.

9. A method as claimed in claims 1-8, characterized in that the compound is a physiologically active compound which, after immobilization on the substrate surface, increases the biocompatibility of the surface.

10. A method as claimed in claim 9, characterized in that the physiologically active compound is heparin or a heparin-analogue.

11. A method as claimed in claim 1, characterized in that the solvent of the solution for activating the compound is of an organic nature.

12. A method as claimed in claim 11, characterized in that the solvent is dioxane, tetrahydrofuran, dimethylformamide, tetrahydrofuran, dimethylformamide, acetone, formamide or dichloroethane.

13. A method as claimed in claims 11-12, characterized in that the solvent is a mixture of a plurality of solvents.

14. A method as claimed in claim 1, characterized in that the substrate surface is cellulose or a cellulose derivative.

15. A method as claimed in claim 1 and claim 8, characterized in that the substrate surface comprises a hydrogel.

16. A method as claimed in claim 1, characterized in that the activated compound is bonded to the substrate surface via a spacer molecule.

17. A method as claimed in claim 16, characterized in that a poly(ethylene oxide)-containing molecule having two or more functional groups is used as the spacer molecule.

18. A method as claimed in claim 17, characterized in that the functional groups are hydroxyl- and/or carboxyl- and/or amino groups.

19. A membrane comprising a surface with modified properties, a compound having been immobilized on the surface via a covalent bond, characterized in that the compound has been immobilized according to the method as claimed in claims 1-18.

20. A membrane as claimed in claim 19, characterized in that the membrane is made of cellulose.

21. A membrane as claimed in claim 19, characterized in that the membrane is made of a cellulose derivative.

22. A membrane as claimed in claims 19-21, characterized in that the compound is a physiologically active compound.

23. A membrane as claimed in claim 22, characterized in that the physiologically active compound is a compound which increases the blood compatibility of the membrane.

24. A membrane as claimed in claim 23, characterized in that the physiologically active compound is a compound comprising a polyelectrolyte.

25. A membrane as claimed in claim 24, characterized in that the physiologically active compound is heparin.

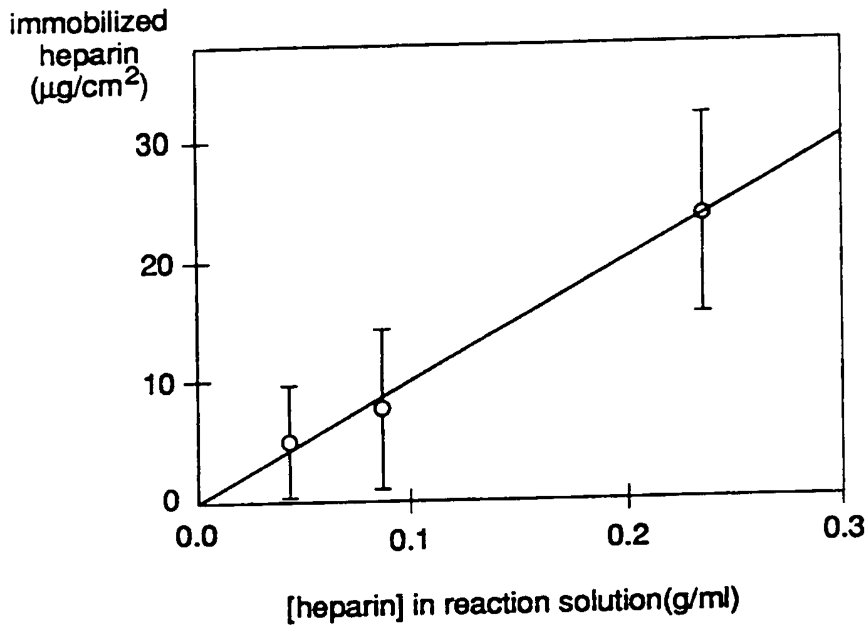
26 A membrane as claimed in claim 24, characterized in that the physiologically active compound is fractionated heparin.

27. A membrane as claimed in claim 24, characterized in that the physiologically active compound is a chemically modified heparin.

28. A membrane as claimed in claim 25, characterized in that the heparin is a heparin having a low molecular weight.

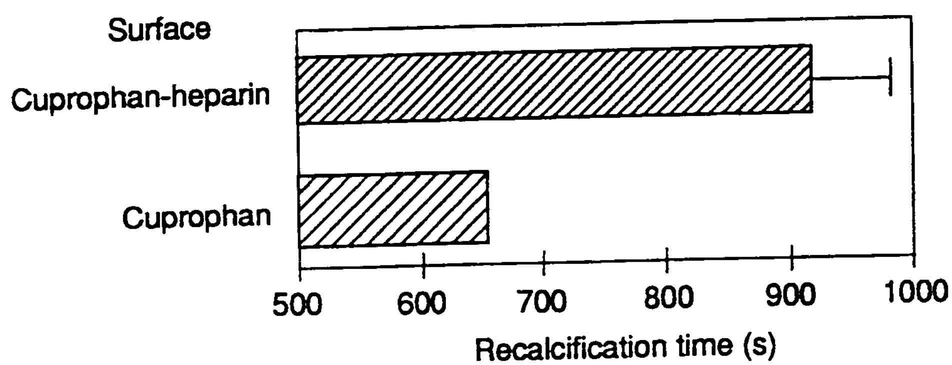
29. A membrane as claimed in claim 25, characterized in that the heparin has an increased activity.

30. A membrane as claimed in claim 24, characterized in that the physiologically active compound is a heparin-analogue.



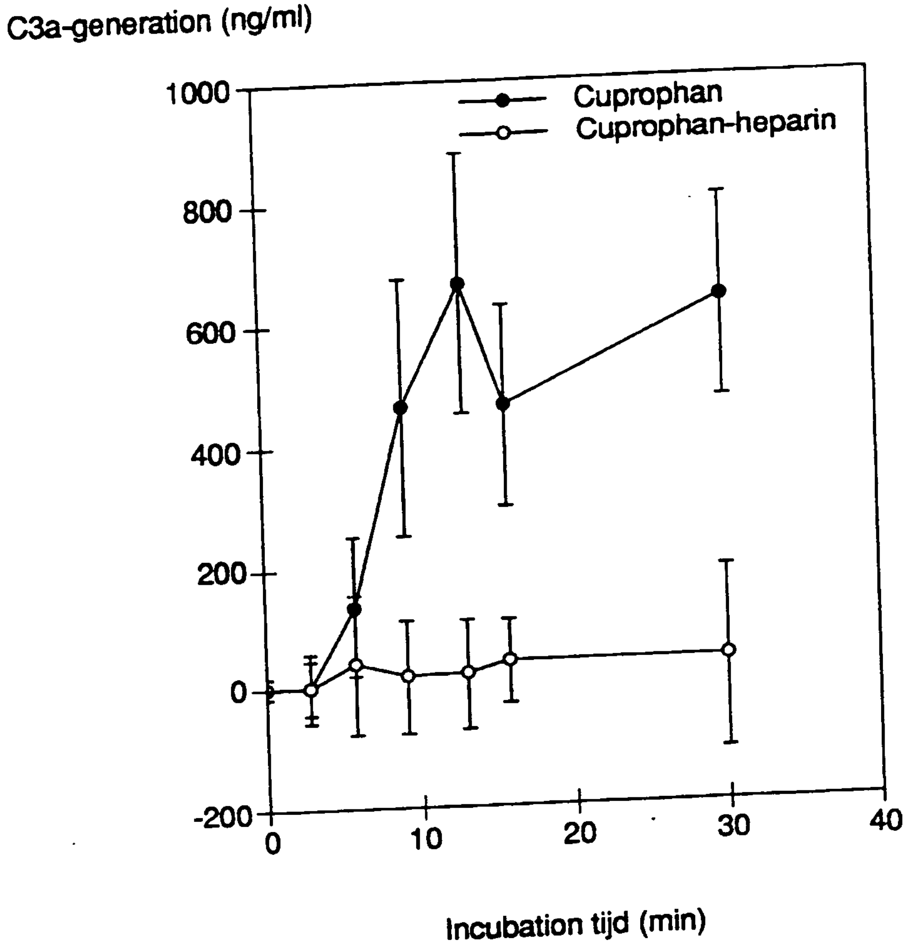
Influence of the heparin concentration of the reaction solution on the amount of immobilized heparin.

FIG. 1



Recalcification time of plasma in contact with heparinized (approx. 25 mg/cm^2) and non heparinized Cuprophan®.

FIG. 2



C3a generation as a result of blood-membrane contact by heparinized (approx. 50 µg/cm^2) and non heparinized Cuprophan® as a function of incubation time.

FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No PCT/NL 91/00103

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61L33/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61L

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	EP,A,46 828 (TEIJIN) March 10, 1982 see page 3, line 5 see page 6, line 25 - line 33 see page 8, line 35 - page 9, line 14 see page 11, line 9 - line 23 see page 16, line 1 - line 15 ---	1
A	EP,A,336 964 (TERUMO K.K.) October 18, 1989 see page 7 - page 9 see claims ---	1-30
A	EP,A,294 905 (SENTRON) December 14, 1988 & NL,A,8 701 337 (SENTRON) January 2, 1989 cited in the application ---	1-30
A	EP,A,86 186 (O.LARM) August 17, 1983 ---	

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
03 OCTOBER 1991	16.10.91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	COUSINS- VAN STEEN

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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-46828	10-03-82	None	
EP-A-336964	18-10-89	JP-A- 63139901	11-06-88
		JP-A- 63154180	27-06-88
		WO-A- 8804183	16-06-88
EP-A-294905	14-12-88	NL-A- 8701337	02-01-89
		JP-A- 2131769	21-05-90
EP-A-86186	17-08-83	AU-B- 562784	18-06-87
		AU-A- 1441583	15-11-84
		CA-A- 1227480	29-09-87
		JP-A- 58147404	02-09-83
		SE-A- 8200751	10-08-83
		US-A- 4810784	07-03-89
		US-A- 4613665	23-09-86

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